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Evaluation of Methyl Blue Sabouraud Dextrose Agar Medium for Differentiation of *Candida dubliniensis* from *Candida albicans*

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ABSTRACT

Background: *Candida dubliniensis* that was first identified as a new species by Sullivan et al. (1995) in Dublin, Ireland (and was subsequently named after its place of origin) while performing an epidemiological investigation of oral candidiasis in HIV-infected and AIDS patients in the early 1990s. This pathogenic Candida species shares many phenotypic features with *Candida albicans* which cause problems its identification. Several phenotypic based tests have been developed to distinguish *C. albicans* from C. *dubliniensis* but none has been demonstrated being sufficient alone for accurate differentiation of the two species.

Aim: To facilitate the differentiation of these species, we evaluated methyl blue Sabouraud dextrose agar medium.

Method: Two hundred Candida spp. were tested including 186 stock strains of *C. albicans* and 14 strains of *C. dubliniensis*. Identification of all these strains was confirmed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using B*In*I (*Avr*II) enzyme. All isolate were inoculated on the medium, incubated at 37 °C in ambient air for 24 to 96h. Examination was done in Fluorescent chamber with illumination at 365 nm.

Result: On this medium, 156 *C. albicans* isolates showed fluorescence at 48h of incubation while none of the 14 *C. dubliniensis* isolates did so even on extending the incubation period. Also after 96h of incubation colonies of all 14 test strains and the two reference strains of *C. dubliniensis* showed yellow colour when viewed against light while others did not.

Conclusion: In conclusion, based the results of our study, methyl blue SDA test offers an additional simple means for identification of *C. dubliniensis*.

Key Words: *Candida dubliniensis, Candida albicans*, Methyl blue Sabouraud dextrose agar medium, Phenotypic identification, Fluorescence

INTRODUCTION

The augmentation of mycoses has been favored by the increased numbers of immunocompromised individuals and species previously not associated with human disease and novel species have been identified as potential pathogens.[1,2,3] A clear paradigm of this phenomenon is *Candida dubliniensis* that was first identified as a new species by Sullivan et al. (1995) in Dublin, Ireland (and was subsequently named after its place of origin) while performing an epidemiological investigation of oral candidiasis in HIV-infected and AIDS patients in the early 1990s.[4,5] The earliest known isolates of *C. dubliniensis* precede the AIDS pandemics with one isolate deposited in the Central Bureau voor Schimmel

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cultures in Holland as *C. albicans* in 1952[6] and another in the British National Collection for Pathogenic Fungi as *C. stellatoidea* in 1957.[4]

Although the first isolate of *C. dubliniensis*, had been recovered way back in 1950s, it was not until the late 1980s or early 1990s that the next isolates of *C. dubliniensis* were identified.[5] This clearly highlights the fact that due to phenotypic similarity with *C. albicans*, *C. dubliniensis* is generally misidentified. Afterwards, *C. dubliniensis* isolates were identified in a wide range of clinical settings.[7,8,9,10,11,12,13] *C. dubliniensis* is most frequently isolated from the oral cavity because of greater adherence to human buccal epithelial cells, mucin, and the oral bacterium Fusobacterium nucleatum. [14]

Although primarily associated with recurrent episodes of oral candidiasis in AIDS and HIV-infected patients, *C. dubliniensis* has also been implicated in cases of superficial and disseminated candidiasis in patients without HIV infection.[4] The incidence of this yeast species is increasing whereas its epidemiology still remains to be elucidated. To gain a more complete understanding of the precise epidemiological role played by *C. dubliniensis* in human disease, it is essential that rapid and reliable tests for its identification be available in routine clinical microbiology laboratory. However, the introduction of such tests has been complicated by the fact that *C. dubliniensis* shares many phenotypic characteristics with *C. albicans*.

A variety of methods have been developed for phenotypic discrimination of isolates of *C. dubliniensis* from *C. albicans*. Variable results have been reported by different authors for each phenotypic method and none has been found sufficient alone for differentiation of the two species.[15] A potentially more stable identification would be one based on the analysis of genetic variability[5] but the tests based on genotypic analysis are not readily applicable for the identification of this species in most average mycology laboratories. So an easy-to-perform phenotypic test, if reliable, would be a valuable tool for differentiation of *Candida dubliniensis* from *Candida albicans*. Therefore, this study was designed to evaluate methyl blue Sabouraud dextrose agar medium for differentiation of the two species.

MATERIALS

This study was conducted in the Mycology Division of Department of Microbiology of a tertiary care hospital in Kashmir, India. The study was approved by the Institute's Ethics Committee.

Test strains

A total of 200 Candida spp. were tested in this study. These included 186 stock strains of *C. albicans* tentatively identified by phenotypic methods such as germ tube formation, colony colour on HiCrome Candida differential agar (HiMedia) and characteristic morphology on corn meal agar. These were isolated from cancer patients with oral candidiasis/colonization and held in stock collection of Mycology Laboratory, Department of Microbiology. Remaining 14 isolates were strains of *C. dubliniensis* which were kindly provided by Dr. Ziauddin Khan (Professor and Chairman Department of Microbiology, Kuwait University). Identification of all these strains was confirmed by Polymerase Chain Reaction-restriction fragment length polymorphism (PCR-RFLP) using *BlnI (AvrII)* enzyme which produced two strong bands of 200 bp and 340 bp in *C. dubliniensis* and only one band of 540 bp in *C. albicans*.[8]

REFERENCE STRAINS

C. albicans 90028 obtained from National Culture Collection of Pathogenic Fungi, Department of Medical Microbiology, PGIMER, Chandigarh and *C. dubliniensis* (type strain CD36) and *C. dubliniensis* (CBS 7987) which were kindly provided by Dr. Ziauddin Khan (Professor and Chairman Department of Microbiology, Kuwait University) were included in the study.

METHODS

Methyl blue SDA was prepared as per manufacturers guidelines. The media was surface inoculated by overnight growth of test as well as reference isolates. Each plate was inoculated with 6 isolates. Incubation was done at 37 °C in ambient air for 24 to 96h. Examination was done in Fluorescent chamber with illumination at 365 nm. [4]

RESULTS

Out of 186 test strains of *C. albicans* 156 strains fluoresced on methyl blue SDA when exposure to long-wave UV light while 30 (16.13%) strains did not. All the test strains of *C. dubliniensis* failed to fluoresce under these conditions at 48h of incubation and even on extending the incubation period further (Figure 1). Also after 96h of incubation colonies of all the test strains of *C. dubliniensis* showed yellow color when viewed against light while none of the test strains of *Candida albicans* formed such colored colonies. (Figure 2) The reference strain of *C. albicans* fluoresced on methyl blue SDA while those of *C. dubliniensis* did not but they showed yellow color when viewed against light while.

DISCUSSION

The aniline dye and the cell wall specific polysaccharides of *C. albicans* react to produces the fluorescent metabolite. No other species of Candida, when grown on SDA with aniline blue medium produces fluorescence when exposed to long-wave ultraviolet light.[16] This property of *C. albicans* has been exploited to differentiate it from *C. dubliniensis*.

In our study we found that fluorescence on methyl blue SDA was 83,87% accurate in identifying *C. albicans* and 100% in identifying *C. dubliniensis.* We also found that 16.1% of *C. albicans* failed to produce fluorescence. Our findings are in agreement with Kirkpatrick WR. et al. (1998) [17] Kantarcıoglu AS et al. (2002)[15] and Akgu¨l O et al. (2009)[18] who found that fluorescence was not visible in all *C. albicans* isolates. Sullivan et al. (1998) reported that fluorescence may not be reproducible in isolates subjected to storage and repeated subculture.[5]

We also found that after prolonged incubation of 96h yellow colouration was produced by the two reference strains of *C. dubliniensis* and not by any of the reference or test strain of *C. albicans*. This feature of *C. dubliniensis* which could be observed by examining the growth against light can also help in the differentiation of *C. dubliniensis* from *C. albicans*. To the best of our knowledge, production of yellow colour by *C. dubliniensis* on methyl blue SDA has not been reported previously. Combining the above two features, methyl blue SDA appears to be a medium well-suited for medical mycological use. It can serve as a primary isolation and differentiation medium for *C. dubliniensis*.

CONCLUSION

In conclusion, methyl blue SDA test offers an additional simple means for identification of *C. dubliniensis*. Our study was first to report production of yellow coloration by colonies *C. dubliniensis* on methyl blue SDA. This feature also differentiates *C. dubliniensis* from *C. albicans* with 100% accuracy. So this medium can be used as a screening medium for identification of *C. dubliniensis.* Further studies over a large number of isolates in multiple laboratories are suggested to evaluate the reproducibility of this simple method.

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COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of Interest: The authors declare that they have no conflict of interest.

Informed consent*:* Informed consent was obtained from all individual participants included in the study.

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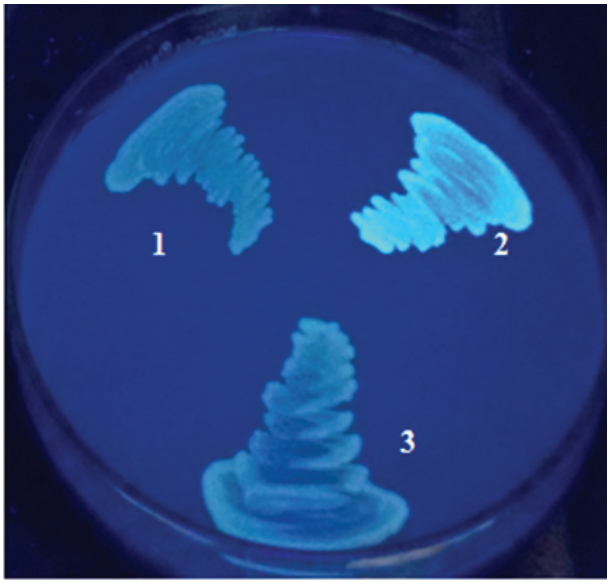

Figure 1: Fluorescence on methyl blue SDA. 1, 3: Strains of *C. dubliniensis*. 2: Strain of *C. albicans*.

Figure 2: Yellow colouration on methyl blue SDA. 1, 3: Strains of *C. dubliniensis*. 2: Strain of *C. albicans.*